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Comparative Tissue Distribution, Biotransformation and Associated Biological Effects by Decabromodiphenyl Ethane and Decabrominated Diphenyl Ether in Male Rats after a 90-Day Oral Exposure Study

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Recent reports indicate that decabromodiphenyl ethane (DBDPE) has become widespread in the environment. Yet databases regarding its bioavailability, biotransformation, and possible toxic effects to wildlife and humans are lacking. In this study, we investigated the bioconcentration and biotransformation of DBDPE after oral exposure and compared the results with those of decabrominated diphenyl ether (BDE-209) using rats as a model. Male rats were orally administrated with corn oil containing 100 mg/kg bw/day of DBDPE or BDE-209 for 90 days, after which the levels of DBDPE and BDE-209 in the liver, kidney, and adipose were measured. Biochemical parameters, including thyroid hormone levels, 13 clinical chemistry parameters, and the mRNA expression levels of certain enzymes were also monitored. Results showed DBDPE was found in all tissues with concentrations 3−5 orders of magnitude lower than BDE-209. At least seven unknown compounds were observed in the DBDPE-exposed rats, indicating that DBDPE biotransformation occurred in rats. These compounds were identified by comparing relative retention times and full-scan mass spectra of DBDPE debrominated products from a photolytic degradation experiment using GC/EI-MS and GC/ECNI-MS analysis. The results showed that debromination of DBDPE to lower brominated BDPEs were not the primary metabolic pathway observed in rats. Two of the metabolites were proposed tentatively as $MeSO_2$-nona-BDPE and $EtSO_2$-nona-BDPE using GC/EI-MS, but their structures require further confirmation by other techniques and authentic standards. In addition, evidence of a biological response to DBDPE and BDE-209 and their metabolites in rats are different. To our knowledge, these results are the first indications for the biotransformation of DBDPE in biota. Further studies are necessary to investigate the metabolites of DBDPE and their mechanisms of toxicities to assess the potential risks of DBDPE.

Introduction

Decabromodiphenyl ethane (DBDPE) and decabrominated diphenyl ether (BDE-209) are two fully brominated flame-retardant chemicals frequently used in industrial applications such as electrical and electronic equipment, wire, textile coatings and blends, and polyester products (*1*). Despite their common use, there is still much debate regarding their environmental fate, behavior, and possible toxic effects on wildlife and humans (*1, 2*). Historically, BDE-209 was thought to be released minimally into the environment during all phases of its use and not available biologically due to its large molecular size and low aqueous solubility. Today, however, it has become a ubiquitous environmental contaminant through-out the world with evidence demonstrating its bioaccumulation and toxicity potential (*2*). One of the most widely discussed and controversial issues surrounding BDE-209's potential for harm is its debromination to potentially more toxic and bioaccumulative constituents within the environment and biota. Several laboratory animal exposure studies have found significant uptake of BDE-209 in fish (*3*), rats (*4*), seals (*5*), and birds (*6*) and demonstrated its capability to metabolically alter to less brominated BDE congeners, primarily octa- and nona-BDE congeners (*3, 4, 6*). Recent studies of BDE-209 have also shown it has potential neurotoxic and neonatal risks (*7*), causes a decrease in epididymal sperm functions (*8*), and disturbs thyroid hormone function (*9*) and hepatic enzyme activity in male mouse offspring (*10*). As a result, the usage of commercial deca-BDE mixture (composed of mainly BDE-209) in electronic equipment (which represents ~80% of total deca-BDE usage) was restricted in the European Union in 2008 (*11*) and voluntarily withdrawn from use in some region, for example, on December 17, 2009, the two U.S. producers of deca-BDE announced commitments to phase out voluntarily deca-BDE in the United States (*12*).

First introduced in the mid-1980s, DBDPE became important commercially as a replacement and alternative to the commercial deca-BDE mixture. Currently there are no figures available on global consumption of DBDPE; however, with concern mounting about BDE-209, the future use of DBDPE is expected to increase. The production of DBDPE in China, for example, where most of the world's electrical equipment is manufactured, has recently shown a growth rate of 80% per year (*13*). Structurally similar to BDE-209, DBDPE is suggested to also behave similarly in the environment and has been detected in a variety of abiotic and biotic environmental samples worldwide. First identification of DBDPE in environmental samples was reported in Sweden in 2004 (*14*), but has since been found in tree bark (*15*), house dust (*16*), birds (*17 and 18*), captive pandas (*19*), and a benthic food web (*20*). More recently, a sharp increasing trend in DBDPE concentrations coupled with a decrease in BDE-209 concentrations was observed in the upper layers of sediment cores collected from an electronic manufacturing region in South China (*21*). This suggests a clear and recent shift from deca-BDE mixture to DBDPE in electronic manufacturing in

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South China. Previous studies have confirmed that DBDPE is a widespread bioavailable contaminant, which can be accumulated in tissues of certain wildlife. Currently, however, databases on the biotransformation and chronic toxicity of DBDPE in animals are lacking. While a few studies have investigated the toxic effects of DBDPE in experimental rats (*22*) and water fleas and exposed zebrafish eggs (*23*), very little information is available on the chronic effects of DBDPE on animals.

In this study, male Sprague−Dawley (SD) rats were orally administrated DBDPE or BDE-209 in corn oil for 90 days. To investigate their bioconcentration and distribution, the levels of DBDPE and BDE-209 in liver, kidney, and adipose were measured after 90 days exposure. The possible degradation/metabolite products of DBDPE in the tissues of the exposed rats were screened by GC/ECNI-MS and GC/EI-MS. Biochemical parameters, including thyroid hormone levels, 13 clinical chemistry parameters, and the mRNA expression levels of certain enzymes were also monitored. The primary objective was to compare the biotransformation and toxic effects of DBDPE and BDE-209 after subchronic exposure using rats as a model animal. Because no authentic standards were available for the identification of DBDPE debromination/metabolic products, we exposed a DBDPE solution (in tetrahydrofuran) to a UV lamp in the laboratory to observe the debromination products of DBDPE. Comparing the GC-MS spectrum profiles in photolytic and metabolic degradation solutions addressed the question whether possible metabolic debromination of DBDPE existed in exposed rats, similar to BDE-209.

Materials and Methods

Chemicals. The chemicals and the recovery and internal standards used in this study are provided in the Supporting Information (SI). We have analyzed various types of samples (sediment, air, soil, house dust, fish, and bird) from Chinese environment, the results indicating that only trace of BDE-77 was found in the highly contaminated sites (such as e-waste sites), and BDE-118, BDE-128, and BDE-181 were not detectable in samples in previous studies (*17, 21*). BDE-77 and -181 were selected as recovery standards and BDE-118 and -128 as internal standards. All solvents and reagents used in these experiments were analytical grade and organic solvents were redistilled using the glass system.

Animals. Male SD rats (21-days-old) obtained from Weitong Lihua Experimental Animal Central (Beijing, China), were separated into three groups (control, DBDPE, and BDE-209 treatments) after a one month acclimation period ($n =$ 12 rats per treatment). The details of the animal exposure conditions are provided in the SI. The doses of 10, 100, 500, and 1500 mg/kg/d -209 were administrated for male mouse from postnatal days 21−70, the results indicating that the decreased epididymal sperm functions in testis were observed in the 500 and 1500 mg/kg/d groups (*8*). The dose level 100 mg/kg/d DBDPE and -209 were hence selected in our study. Liver and kidney of rats were selected to investigate the metabolic potentials and the metabolic products are thought to be enriched in those organs. The adipose tissue was thought to be a target store of hydrophobic organic compounds due to its high lipid content. All rats were euthanized after 90 days exposure. Serum, liver, kidney, and adipose were immediately collected from each rat and tissue samples were frozen in liquid nitrogen and then stored at −20 or −80 °C until used for chemical analysis and toxic experiments, respectively.

DBDPE Photolytic Degradation. Preparation of the DBDPE solution for the photolytic degradation experiment is provided in the SI. Only debrominated congeners were observed and identified.

Sample Extraction and Analysis. Details of the analytical procedures are described elsewhere (*19*). The complete analysis conditions of the solution used in the photolytic degradation experiment and extractions of selected tissue samples from DBDPE exposed rats are provided in the SI.

QA/QC and Data Analysis. Procedural blanks covering the whole procedure were run in parallel with the samples on each batch of extraction. Blind triplicate samples, triplicate spiked blanks, and triplicate spiked matrices were performed throughout the study. While BDE-209 was found in procedural blanks, levels were significantly ($p < 0.05$) lower than the levels in the control rat samples. The BDE-209 sample concentrations were corrected from background concentrations of BDE-209 by subtracting three times the mean BDE-209 level in the blanks. Details of QA/QC are given in the SI. Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL) for comparisons of means between and within treatment groups. Significance was set at $p < 0.05$. Instrumental QC included regular injections of solvent and standard solutions.

Thyroid Hormone and Serum Parameter Analysis. The levels of thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), in serum were monitored by radioimmunoassay. Blood collected from the control and exposed groups at the same time to avoid the fluctuation of thyroid hormone levels during one day. Standard spectrophotometric methods for the HITAC7170A automatic analyzer were used to measure 13 serum parameters including aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol (T-CHO), high density lipid-cholesterol (HDL-C), creatinine (Cr) and total bile acids (TBA) (details provided in the SI). Differences between the control and treatment groups were determined using a one-way analysis of variance (ANOVA). The calculation was based on discrimination significance between classes at the level of $p < 0.05$.

Gene Expression Analysis. Total RNA from the individual liver samples were isolated by the TRIzol reagent (Invitrogen Corp., Carlsbad, CA) in accordance with the manufacturer's instructions. The concentration of RNA was measured by absorbance at 260 nm performed in a UV1240 spectrophotometer (Shimadzu, Japan), and 260/280 nm absorbance ratio for estimating its purity. The details of cDNA preparation and condition of real time polymerase chain reaction are provided in the SI. The expression levels of selected genes and their PCR primers are also given in SI Table S1. Every sample was analyzed in triplicate. The housekeeping gene β-actin was used as an internal control, and quantification of the transcripts was performed by the $\Delta\Delta C_T$ method (*24*).

Results and Discussion

DBDPE and BDE-209 in Rat Tissues. The concentrations of DBDPE and BDE-209 were both elevated in the tissues of dosed rats compared to the control groups (Figure 1). The control tissues contained low amounts of DBDPE (0.38−13 ng/g lw) and relatively high levels of BDE-209 (103−2186 ng/g lw) due to background exposure (e.g., feed or air-borne dust). The DBDPE concentrations in the tissues of DBDPE-exposed rats, however, were elevated 13 to 1444 folds over the control levels, and the tissues of BDE-209-exposed rats contained BDE-209 at concentrations 3 orders of magnitude higher than those in control rats. The concentrations of BDE-209 in tissues (637 365 ± 103 in kidney, 1986, 152 ± 104 in liver, and 143 075 ± 112 ng/g lw in adipose, respectively) of BDE-209-exposed rats were 3−5 orders of magnitude higher than the DBDPE concentrations (11.2 ± 0.01 in kidney, 177 ± 111 in liver, and 549 ± 48 ng/g lw in adipose tissue, respectively) of DBDPE-exposed rats. This indicates that the extent of bioavailability and/or bioaccumulation of DBDPE were lower than for BDE-209, which was not surprising given that DBDPE has lower solubility (*1, 14*). The concentration

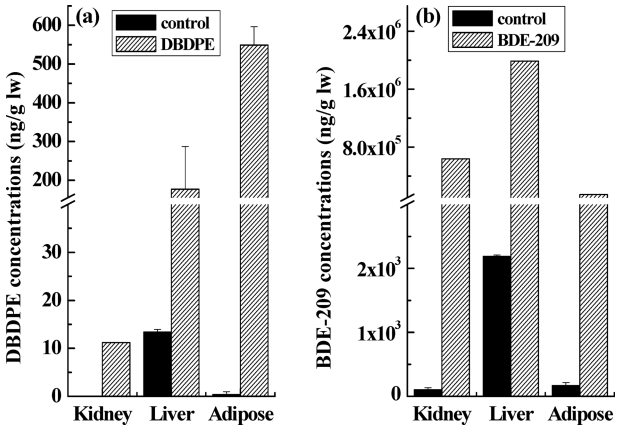

FIGURE 1. DBDPE and BDE-209 concentrations (ng/g lipid weight) measured in the kidney, liver, and adipose of the exposed 100 mg/kg/d DBDPE (a) or BDE-209 (b) including comparison of DBDPE and BDE-209 levels measured in control rats. Values represent the mean and standard deviation of six rats per group.

of BDE-209 in the livers of BDE-209-exposed rats was substantially higher than those of the other two tissues on a lipid weight (Figure 1b). The preferential accumulation of BDE-209 in the liver rather than other tissues has also been observed in studies of BDE-209 in rats (*4*) and fish (*3*). In the DBDPE-dosed rats, however, adipose displayed the greatest accumulation of DBDPE after 90-days exposure (Figure 1a). The disposition of DBDPE, therefore, appears to be different from that of BDE-209. This difference may be due to differences in the toxicokinetic properties of the two compounds in high-dosed, subchronic exposed rats or the different capacity for binding affinity of DBDPE and BDE-209 to different tissues. It is worth noting that the liver in the control rats, which could represent the environmentally relevant level of exposure, consistently had the highest concentrations of both DBDPE and BDE-209 among the three tissue compartments, suggesting that the disposition of DBDPE in rat tissues appeared to be dose dependent. Future studies are needed to examine the tissue distribution of DBDPE within an organism.

Biotransformation. The focus of the current study was to screen and identify possible metabolites in DBDPE-exposed rats. Compared to the chromatograms of the control rat tissues, several peaks of unknown, bromine-containing compounds eluting before DBDPE were observed in DBDPE-exposed rat tissues. Besides DBDPE, at least seven unknown compounds marked as n^* ($n = 1-7$, Figure 2b) were specifically identified in the liver of the DBDPE-exposed rats. In Figure 2b, the unmarked peaks were the PBDEs (BDE-202, -197, -208, -207, -206, and -209, respectively), which were also observed in the control group (Figure 2d), and might be derived from the background exposure of commercial mixture of BDE-209 or its metabolites. The presence of these unknown brominated compounds in the DBDPE-exposed rats indicates that DBDPE metabolism does occur in rats. To determine whether debrominated metabolites of DBDPE arose in the exposed experiment, a DBDPE photodegradation experiment was conducted to obtain the debrominated products of DBDPE. Irradiation of the DBDPE solution under UV lamp produced a number of peaks, identified as hepta- to nona-BDPE congeners by their GC/EI-MS full scan mass spectra (SI Figure S1). None of the unknown brominated compounds (peaks 1* to 7*, Figure 2b) in exposed rats, however, had the same retention time as those compounds observed in the photodegradation experiment, where DBDPE debrominated to lower brominated BDPEs. This suggests that reductive debromination of

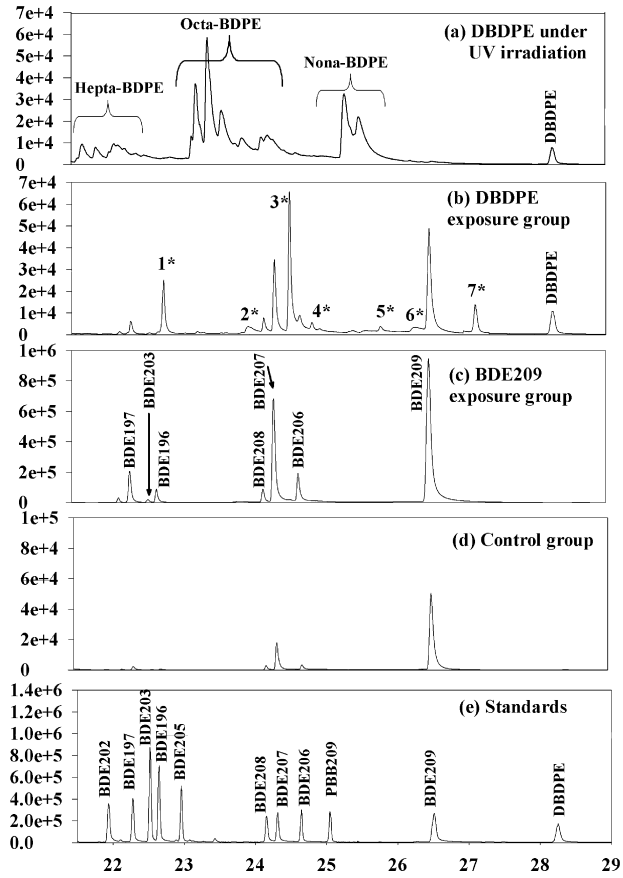

FIGURE 2. Comparison of GC/ECNI-MS chromatogram (*m/z* 79 and 81), (a) DBDPE under UV irradiation; (b) extracts of liver tissues of a 100 mg/kg/d DBDPE-exposed rat; (c) extracts of liver tissues of a 100 mg/kg/d BDE-209-exposed rat; (d) extracts of liver tissues of a control rat; (e) standards.

DBDPE to lower brominated BDPEs is not likely to be a substantial metabolic pathway in rats.

To further identify the unknown peaks found in the exposed rats, selected liver extracts were concentrated into a smaller volume and were analyzed in both EI and ECNI to record the full scan mass spectra of these unknown substances. Due to the low concentrations and large interferences, only two DBDPE metabolites corresponding to peaks 3* and 7* had clear/visible ion fragmentation patterns to provide structural information (Figure 3 and SI Figure S2). In the ECNI mode, the bromine ions (m/z 79, 81, 160, 162) were the dominant fragments for peaks 3* and 7* (SI Figure S2). The fragments with a low abundance in ECNI mode were also recorded by the EI mode. The mass spectra of both peaks did not match with any spectra available for DBDPE or its debrominated products in the GC/EI-MS analyses. The dominating fragments in DBDPE and its debrominated products were ion fragment clusters corresponding to the cleavage of the ethyl bond (SI Figure S1). Peak 3* yielded an intense fragment ion of m/z 809.5 as a base peak, however, which indicated of the loss of two bromine atoms from the molecular ion at m/z 969.3 (Figure 3a). The ion fragment clusters observed in this unknown peak all differ by 80 m/z units, corresponding to the successive loss of one bromine atom or a fragment with m/z ~80. Although a similar molecular ion cluster to the parent DBDPE (m/z 971) was present in peak 3* (m/z 969.3), their retention times in the GC chromatogram were different, with much earlier elution for peak 3* (Figure 2b). The relative elution order of peak 3* (eluting after BDE-207 and between octa-BDPEs and nona-BDPEs) suggests that only one or two bromine atoms in a ring of DBDPE had been substituted and the substituting

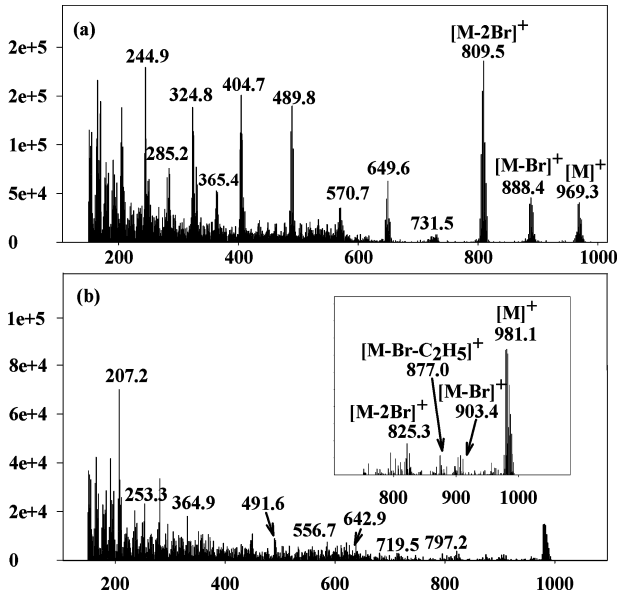

FIGURE 3. GC/EI-MS full-scan spectra of two DBDPE metabolites corresponding to the peaks 3* and 7* detected in livers of male rats exposed to 100 mg/kg/d DBDPE for 90 days using a Thermo TRACE GC coupled to a DSQII MS system.

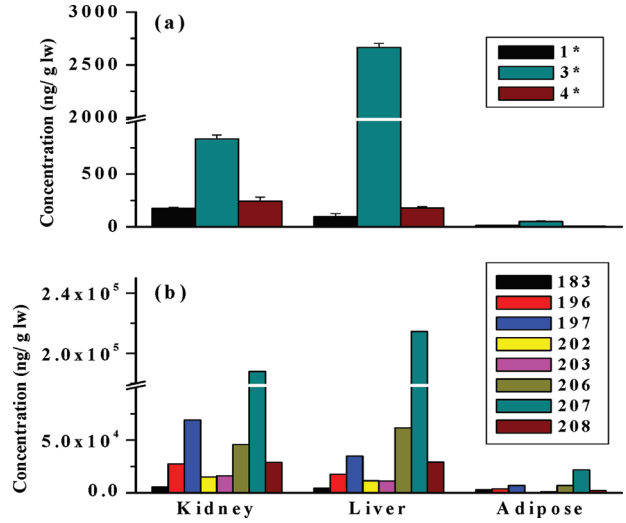

FIGURE 4. Metabolites of DBDPE and BDE-209 in different tissues of male rats exposed to 100 mg/kg/d DBDPE (a) or BDE-209 (b) for 90 days. Values are presented as the mean ± SEM for six rats per group.

group could have had an ion fragment cluster at m/z~80. A methyl sulfone (SO_2CH_3 or $MeSO_2$) group with ion at m/z 79 can be placed at the substituted position and a $MeSO_2$-substituted nona-BDPE would be hypothesized for peak 3* (SI Figure S3a). The most abundant fragment (m/z 809.5) in peak 3* may also result from the loss of the $MeSO_2$ group plus a bromine atom $[M-(SO_2CH_3+Br)]^+$, and a $[M-SO_2CH_3]^+$ ion may also contribute to the fragment of m/z 888.4. The $MeSO_2$ metabolites of chlorinated aromatic compounds, for example, polychlorinated biphenyls (PCBs), polychlorobenzenes, 1,1-bis(chlorophenyl)-2,2-dichloroethene (DDE), and polychloroterphenyls, have been observed in many laboratory exposure studies and various wildlife (*25*). No ion fragments corresponding to $[M-CH_3]^+$ and/or $[M-xBr-CH_3]^+$ (x = the number of bromine atoms) were observed, however, to further confirm the presence of $MeSO_2$- substituted metabolites in our study. In studies on the $MeSO_2$-PCBs, characteristic ions at $[M-(SO_2CH_3+Cl)]^+$ and $[M-SO_2CH_3]^+$ were also observed as major fragment ions depending on the substitution position of the $MeSO_2$. Only weak or no $[M-CH_3]^+$ ion was present for meta- and para- or ortho-$MeSO_2$-substituted PCBs (*26*).

Peak 7* gave a molecular ion at m/z 981.1 as a base peak in its mass spectrum, instead of a fragment ion of $[M-xBr]^+$, and showed a different fragmentation pattern from peak 3* (Figure 3b). Ion fragment clusters at 903.4 $[M-Br]^+$, 877.0 $[M-Br-C_2H_5]^+$, 825.3 $[M-2Br]^+$, and 797.2 $[M-2Br-C_2H_5]^+$ were also detected but with low abundances as compared with molecular ion, indicating that this unknown compound has an ethyl substitution. An ethyl sulfone ($EtSO_2$)-substituted nona-BDPE could, therefore, be hypothesized for peak 7* if DBDPE can be metabolically transformed to sulfur-containing metabolites (SI Figure S3b). Although we have provided conjecture for the structure of these two unknown DBDPE metabolites, it is not possible to confirm their identification due to a lack in standards for DBDPE derivatives. Further studies are warranted to determine the structure of DBDPE metabolic products. Among seven potential metabolic products of DBDPE, three products (peaks 1*, 3*, and 4*) were detected consistently in all tissue samples of the DBDPE-exposed rats using a GC/ECNI-MS monitoring ions 79 and 81. To semiquantify the levels of these metabolic products, it was assumed the relative response factors for the three unknown metabolites were similar to that of BDE-196, -207, and -206, which were eluting close to peaks 1*, 3*, and 4* in the GC/ECNI-MS chromatogram, respectively (Figure 2). For metabolite 3*, the tissue distributions in order of concentrations were liver > kidney > adipose, whereas the order for metabolites 1* and 4* was kidney > liver > adipose (Figure 4a). Other studies have demonstrated that BDE-209 is reductively debrominated to lower brominated congeners in dosed fish and rats, with the major debrominated metabolites being octa- and nona- BDEs (*3, 4, 6*). In the present study, the same results were obtained in BDE-209 exposed rats where BDE-207 and -197 were the major isomers in the nona- and octa- homologue groups, as a possible result of meta-debromination(s) of BDE-209 (Figure 4b). The dominant product, BDE-207, generally accumulated in tissues in the order, liver > kidney> adipose. While BDE-203, -202, -197, -196, and -183 generally accumulated in the kidney.

The concentrations of all metabolic products in the liver and kidney of dosed-DBDPE or BDE-209 rats were 2−53 fold higher than those of the adipose on lipid weight basis, implying a high preference of these metabolites for liver and kidney storage. Preferential storage of hepta- to nona-BDEs in the liver rather than adipose has been reported in administrated fish (*3*) and rats (*27*) and wild terrestrial mammals (*28*). A number of studies measuring distribution of multiple $MeSO_2$-PCBs into various tissues in biota have also demonstrated that liver plays the predominant role relative to other tissue compartments such as fat and muscle (*29*). Preferential binding to blood proteins of higher brominated BDEs or $MeSO_2$-PCBs may be one of the mechanisms underlying the hepatic sequestration of these compounds.

Clinical Chemistry Parameters. No significant changes in body, liver and kidney weight, or relative liver and kidney weight were observed following exposure to 100 mg/kg/d DBDPE or BDE-209 compared with controls. This indicated that these dose levels did not cause overt toxicity (SI Table S2).

The DBDPE and BDE-209 induced hepatotoxicity in rats were indicated by the serum clinical chemistry data for AST, ALP, T-CHO, HDL-C, Cr, and TBA (SI Table S3). The significant alterations in the DBDPE group included a decrease in Cr levels and AST and ALP activities and an elevation in TBA levels ($p<0.05$), indicating possible oxidative stress due to the accumulation of DBDPE or its metabolites (*30*). In the BDE-209 group, T-CHO, HDL, and TBA levels were increased significantly ($p < 0.05$) (SI Table S3). The

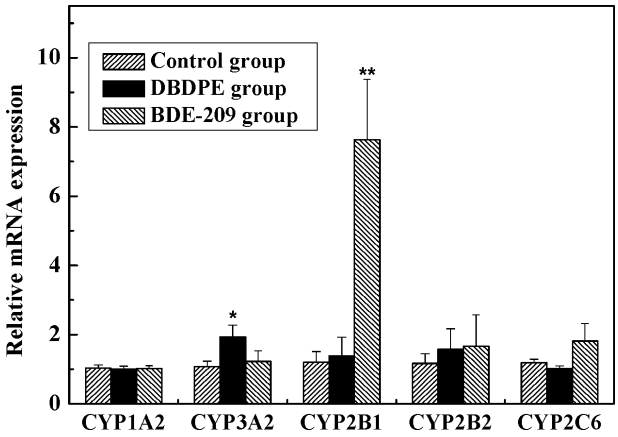

FIGURE 5. Relative liver mRNA expression of CYP1A2, 3A2, 2B1, 2B2, and 2C6 from control and 100 mg/kg/d DBDPE-exposed or BDE-209-exposed rats (mean ± SEM; $n = 6$). Statistically significant differences between controls and treatments are indicated by * for $p < 0.05$, and ** for $p < 0.01$.

observed increase in T-CHO and HDL-C levels, which both participate in fat metabolism, imply that BDE-209 and the debrominated metabolites probably disrupt fat metabolism in subchronic exposure.

To investigate the potential influence of the two compounds and their metabolites on endocrine-related processes, thyroid hormone levels in serum were measured by radioimmunoassay. For T_3 levels, significant increases were observed in the DBDPE treatment ($p < 0.05$) (SI Table S3). Compared to the control group, the T_4 levels of the two treatments were neither statistical significantly ($p > 0.05$). Many researchers have demonstrated that PBDEs (*31, 32*), phenolic PCB metabolites (*32*), and some $MeSO_2$-CBs (*25*) can disturb thyroid systems, shown mainly through reduced thyroid hormone levels in experimental animal models and several in vitro test systems. In the present study, BDE-209 had no effect on the two thyroid hormone levels, while DBDPE increased the T_3 level. It is worth noting, however, that DBDPE can alter thyroid hormone homeostasis. Thus, further studies are required to determine this mechanism and its importance from a toxicological point of view.

The mRNA Expression Levels of Certain Enzymes. The cytochrome (CYP) monooxygenases are a group of proteins responsible for the oxidation of drugs, environmental pollutants, and endogenous compounds (*33*). The mRNA expressions of genes encoding these enzymes were investigated in each group of rats to determine possible biological activities of DBDPE and BDE-209, as well as their metabolites. Compared to the control group, significant increases occurred in the expression of CYP3A2 in the DBDPE treatment for 1.24-fold ($p < 0.05$) (Figure 5), and in the expression of CYP2B1 in the BDE-209 group for 7.34-fold (Figure 5) ($p < 0.001$). The expression of CYP1A2, CYP2B2, and CYP2C6, however, showed only slight and nonsignificant increase or decrease ($p > 0.05$). In the present study, CYP3A2 was significantly up-regulated after exposure to DBDPE, which could explain why sulfur-containing metabolites were found in exposed rats. Additionally, CYP2B1 was significantly up-regulated indicating BDE-209 and/or its metabolites probably induced the CYP2B1 mRNA expression. The inductions of CYP3A2 and CYP2B1 after DBDPE and BDE-209 exposure, respectively, provided evidence of a biological response to DBDPE and BDE-209 at the single dose level used in our study.

In conclusion, the present study demonstrates that both DBDPE and BDE-209 are bioaccumulative and biotransformative in male rats, although the DBDPE concentration in tissues was significantly lower than the BDE-209 concentrations. Significantly, several probable metabolites of DBDPE were observed in male rats. Unlike BDE-209, however, reductive debromination to lower brominated BDPEs does not appear to be the primary metabolic mechanism of DBDPE in rats. Two of the unknown metabolites were tentatively proposed as $MeSO_2$-nona-BDPE and $EtSO_2$-nona-BDPE with GC/EI-MS but further confirmation by other techniques and authentic standards is required. Furthermore, evidence of a biological response to DBDPE or BDE-209 and their metabolites in rats are different. Further studies are necessary to fully confirm the metabolites of DBDPE and their mechanisms of toxicities to determine whether DBDPE should be considered a suitable alternative for PBDEs.

Acknowledgments

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Supporting Information Available

Table S1 shows the sequences of primers used for real-time RT-PCR amplification. Body weight and absolute and relative liver and kidney weights of rats treated with DBDPE and BDE-209 are given in Table S2. Table S3 shows the effect of DBDPE and BDE-209 on selected clinical chemistry parameters and the thyroid hormone levels. Figure S1 gives the mass spectra of DBDPE, nona-BDPE and octa-BDPE produced by irradiation of the DBDPE solution. Ion fragmentation patterns in EI and ECNI for two DBDPE metabolites corresponding to the peak 3* and 7* in Figure 2 are shown in Figure S2 and S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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